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Synthesis, Crystal Structure and Biological Activity of 1-Cyano-*N*-(2,4-dichlorophenyl)cyclopropanecarboxamide

YONG-LAI XUE[1], YONG-GANG ZHANG[3] and XING-HAI LIU[2,*]

[1]School of the Environment, Jiangsu University, Zhenjiang 212013, P.R. China
[2]College of Chemical Engineering and Material Science, Zhejiang University of Technology, Hangzhou 310014, Zhejiang, P.R. China
[3]Institute of Microbe, Shandong Academy of Science, Jinan, P.R. China

*Corresponding author: E-mail: xhliu@zjut.edu.cn

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A cyclopropane derivative, 1-cyano-*N*-(2,4-dichlorophenyl)cyclopropanecarboxamide ($C_{11}H_8N_2OCl_2$) was synthesized and its structure was studied by X-ray diffraction, FTIR, ^{1}H NMR, MS and elemental analysis. The crystals are monoclinic, space group C2 with a = 14.387(9), b = 6.926(4), c = 12.237(7) Å, α = 90.00, β = 100.386(10), γ = 90.00°, V = 1199.4(12) Å^3, Z = 4, F(000) = 520, D_c = 1.413 g/cm^3, μ = 0.520 cm^{-1}, the final R = 0.0603 and wR = 0.1653. A total of 2976 reflections were collected, of which 1134 were independent (R_{int} = 0.0381). The preliminary biological test showed that the synthesized compound is bioactive against the KARI of *Escherichia coli*.

Key Words: Synthesis, Crystal structure, Biological activity, 1-Cyano-*N*-(2,4-dichlorophenyl)cyclopropanecarboxamide.

INTRODUCTION

Ketol-acid reductoisomerase (KARI; EC 1.1.1.86) is an attractive target for agro-chemical and medicinal discovery because it catalyzes the second important step in the biosynthesis of the branched chain amino acid[1]. The KARI exists in microorganisms and plants, not in mammals. Thus it is an ideal target from which to design non-toxic KARI-inhibitors as potential novel drugs.

Some commercial pesticides which inhibit the first enzyme as acetohydroxyacid synthase, have been successfully developed. For example, sulfonylureas[2] are a series of herbicides which inhibit ALS. It has stimulated the research into inhibitors of other enzymes in the pathway, including the second enzyme[3], ketol-acid reductoisomerase (KARI; EC 1.1.1.86). The reaction catalyzed by KARI is shown in Fig. 1, which consists of two steps[4,5], an alkyl migration followed by a NADPH dependent reduction. Until now, only HOE 704[6], IpOHA[7], 1,2,3-thiadiazoles[8] and CPD derivatives[9] were shown to be potential inhibitors targeting KARI (Fig. 1).

In view of these facts mentioned above and also as a part of our work on the synthesis of bioactive lead compounds for crop protection, the 1-cyano-*N*-(2,4-dichlorophenyl)-cyclopropanecarboxamide was designed and synthesized. The biological activity of the compound is also determined.

EXPERIMENTAL

Melting points determined by a Yanaco MP-241 apparatus and uncorrected. Infrared spectra were recorded on a Bruker Equinox55 spectrophotometer as KBr tablets. ^{1}H NMR spectra were measured on a Bruker AC-P500 instrument (300 MHz) using TMS as internal standard and $CDCl_3$ as solvent. Mass spectra were recorded on a Thermo Finnigan LCQ advantage LC/mass detector instrument. Crystallographic data of the compound collected on a Rigaku Saturn CCD diffractometer. All chemicals were of AR grade.

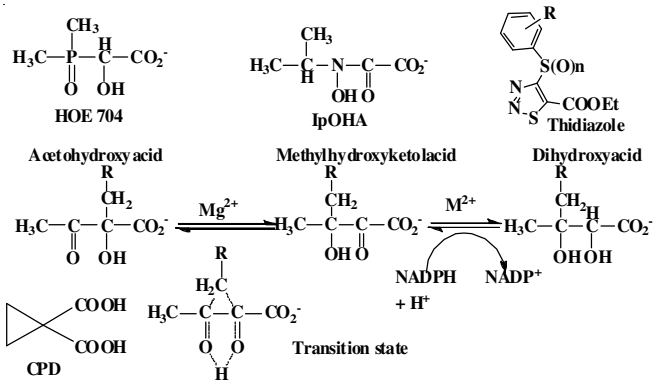
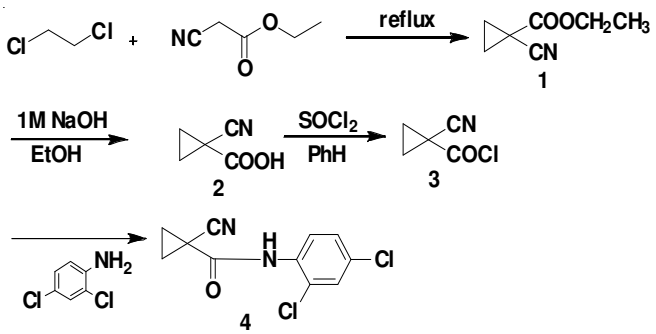


Fig. 1. Reaction catalyzed by KARI

Crystal structure determination: The crystal of title compound with dimensions of 0.24 mm × 0.22 mm × 0.16 mm was mounted on a Bruker CCD area-detector diffractometer with a graphite-monochromated MoK_α radiation ($\lambda = 0.71073$ Å) by using a phi and scan modes at 294(2) K in the range of $1.6° \leq \theta \leq 25.0°$. The crystal belongs to monoclinic system with space group C^2/m and crystal parameters of a = 14.387(9) Å, b = 6.926(4) Å, c = 12.237(7) Å, $\alpha = 90°$, $\beta = 100.386(10)°$, $\gamma = 90°$, V = 1199.4(12) A^3, $D_c = 1.413$ g/cm^3. The absorption coefficient $\mu = 0.520$ mm^{-1} and Z = 4. The structure was solved by direct methods with SHELXS-97[10] and refined by the full-matrix least squares method on F^2 data using SHELXL-97. The empirical absorption corrections were applied to all intensity data. H atom of N-H was initially located in a difference Fourier map and were refined with the restraint Uiso(H) = 1.2 Ueq(N). Other H atoms were positioned geometrically and refined using a riding model, with d(C---H) = 0.93-0.97 Å and Uiso(H) = 1.2 Ueq(C) or 1.5 Ueq (Cmethyl). The final full-matrix least squares refinement gave R = 0.0603 and wR = 0.1653.

Synthesis: Ethyl cyanoacetate (22.6 g, 0.2 mol), 1,2-dichloroethane (160 g, 0.2 mol), potassium carbonate (220 g, 1.6 mol) and catalytic amount of Bu_4NHSO_4 (1.0 g) were vigorously refluxed in 1,2-dichloroethane for 6 h after, which the reaction mixture was poured into water (800 mL). The product was extracted with ether (5 × 100 mL), combined extracts were dried over $MgSO_4$ then the solvent was removed on a rotary evaporator and the reside was distilled under pressure: b.p. 115-118/15 mmHg. An ester (0.03 mol) was added to a *ca.* 15 % aqueous solution containing 3 mol equivalents of sodium hydroxide and a suspension was vigorously stirred at ambient temperature for 2 days until a homogenous solution was formed. The solution was extracted with ether (2 × 50 mL) to remove traces of unreacted ester, the water phase was acidified with conc. HCl and a free acid was extracted with ether (3 × 100 mL). The combined extracts were dried over $MgSO_4$ then the solvent was removed on a rotary evaporator (Yields 51 %). To a benzene solution (25 mL) of cyanocyclopropanecarboxylic acid (7.50 mmol) was added thionyl chloride (30 mmol) and the mixture was refluxed for 2 h to give acid chloride. Then dropwised the acid chloride to 2,4-dichloroaniline (7.50 mmol), then vigorously stirred at ambient temperature for 4 h (**Scheme-I**). The yield was 73.4 % with m.p. 99-100 ºC. ^{1}H NMR ($CDCl_3$, 300 M) 1.63-1.82 (m, 4H, CH_2), 7.28 (d, *J* = 2.270 Hz, 1H, ArH), 7.44 (d, *J* = 2.297 Hz, 1H, ArH), 7.28 (d, *J* = 8.888 Hz, 1H, ArH), 8.67 (s, 1H, NH); IR (cm^{-1}) 3398, 3115, 2236, 1699, 1583, 1510, 959, 923, 821, 727. ESI-MS: 253.29, 185.98, 149.81, 114.05. Elemental analysis: C, 51.70; H, 3.21; N, 10.79; calculated from $C_{11}H_8N_2OCl_2$. Observed: C, 51.79; H, 3.16; N, 10.98.



Scheme-I: Synthesis route of the title compound

Biochemistry of KARI

Cloning, expression and purification of rice KARI: The KARI resultant expression plasmid was obtained from the Prof. Ronald G. Duggleby's lab[9] and was used to transform *Escherichia coli*. BL21(DE3) cells. A single colony of these cells was inoculated into 20 mL of LB medium containing 50 mg/mL kanamycin. The culture was incubated overnight at 37 ºC and was used to inoculate each of two 1000 mL volumes of LB medium containing 50 mg/mL kanamycin; the cultures were incubated at 37 ºC with shaking. When an OD600 of 0.6 was reached, expression was induced by adding 1 µL isopropyl β-D-thiogalactoside to each culture; these were then incubated at room temperature (37 ºC) for a further 2 h with shaking and the cells were harvested by centrifugation and were keep in-30 ºC.

The frozen cell pellet was thawed, suspended in ice-cold purification buffer [50 mM *Tris*-HCl (pH 7.9)/500 mM NaCl] containing 5 mM imidazole and then treated with lysozyme (10 mg/g of cells for 0.5 h at 0 ºC). The cells were disrupted by sonication, insoluble material was removed by centrifugation and the supernatant was passed through a 0.45 mm filter. The cell extract was applied to a 7 mL column of His·Bind resin (Novagen) that had been charged by using 50 mM $NiSO_4$ then equilibrated with purification buffer containing 5 mM imidazole. The loaded column was washed with 23 mL of the same buffer, followed by 30 mL of purification buffer containing 25 mM imidazole and then KARI was eluted with 30 mL of purification buffer containing 1 M imidazole. Fractions containing the enzyme were pooled, concentrated to 2.5 mL by ultrafltration and exchanged into 20 mM Na-Hepes buffer, pH 8.0 using a Pharmacia PD-10 column. The eluate was snap-frozen in low-temperature refrigerator and stored at -78 ºC.

Enzyme and protein assays(*in vitro*): Gerwick *et al.*[11] reported that the inhibition of *Escherichia coli* KARI is time-dependent. KARI activity was measured by following the decrease in A_{340} at 30 ºC in solutions containing 0.2 mM NADPH, 1 mM $MgCl_2$, substrate 2-acetolactate and inhibitors as required, in 0.1 M phosphate buffer, pH 8.0. Inhibitors was preincubated with enzyme in phosphate buffer at 30 ºC for 10 min before the reaction was started by adding the substrate combining with NADPH and $MgCl_2$. Protein concentrations were estimated using the bicinchoninic acid method[12] and protein purity was assessed by SDS-PAGE[13]. The yield of recombinant rice KARI from a 30 culture was 50 mg with a specific activity (measured with saturating 2-acetolactate) of 1.17 U/mg. The 2-acetolactate was prepared by us.

Supplementary material: CCDC 832506 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge crystallographic data centre (CCDC), 12, Union Road, Cambridge CB2 1EZ, UK (Fax: + 44-1223-336033; email: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

TABLE-1
SELECTED BOND LENGTHS (Å), ANGLES (º) and TORSION ANGLES (º) FOR THE TITLE COMPOUND

Bond lengths	Å	Bond angles	º	Torsion angles	º
Cl(1)-C(10)	1.732(5)	C(4)-N(2)-C(5)	127.9(4)	C(1)-C(2)-C(3)-N(1)	-147.1(3)
Cl(2)-C(8)	1.751(5)	C(3)-C(2)-C(4)	117.3(4)	C(5)-N(2)-C(4)-O(1)	0
O(1)-C(4)	1.205(6)	C(4)-C(2)-C(1)	116.0(4)	C(5)-N(2)-C(4)-C(2)	180
N(1)-C(3)	1.140(7)	N(1)-C(3)-C(2)	177.4(6)	C(3)-C(2)-C(4)-O(1)	180
N(2)-C(4)	1.352(6)	O(1)-C(4)-N(2)	124.7(4)	C(1)-C(2)-C(4)-O(1)	-32.2(3)
N(2)-C(5)	1.414(6)	O(1)-C(4)-C(2)	120.3(4)	C(1)-C(2)-C(4)-N(2)	147.8(3)
C(1)-C(2)	1.533(6)	N(2)-C(4)-C(2)	115.0(4)	C(4)-N(2)-C(5)-C(6)	0
C(2)-C(3)	1.423(8)	C(6)-C(5)-C(10)	118.1(4)	C(4)-N(2)-C(5)-C(10)	180
C(2)-C(4)	1.491(7)	C(6)-C(5)-N(2)	124.6(4)	C(10)-C(5)-C(6)-C(7)	0.000(1)
C(5)-C(6)	1.382(6)	C(10)-C(5)-N(2)	117.2(4)	N(2)-C(5)-C(6)-C(7)	180
C(5)-C(10)	1.403(6)	C(5)-C(6)-C(7)	122.2(4)	C(6)-C(7)-C(8)-Cl(2)	180
C(6)-C(7)	1.384(7)	C(7)-C(8)-Cl(2)	118.9(4)	N(2)-C(5)-C(10)-C(9)	180
C(7)-C(8)	1.396(7)	C(5)-C(10)-Cl(1)	120.2(4)	C(6)-C(5)-C(10)-Cl(1)	180

RESULTS AND DISCUSSION

The IR spectrum of the title compound tested shows absorption bands at 3398 cm^{-1} originating from the stretching vibration of NH. The strong band at 2236 cm^{-1} can be assigned to the CN strentching vibration. The strong band at 1699 cm^{-1} can be assigned to the C=O strentching vibration. The absorption of the phenyl ring is at 1583, 1510 cm^{-1}. The MS of title compound is ion peak.

Structure of the title compound: The selected bond lengths, bond angles and torsion angles listed in Table-1 respectively. The molecular structure and atom labels are shown in Fig. 2. The π-π stacking is shown in Fig. 3.

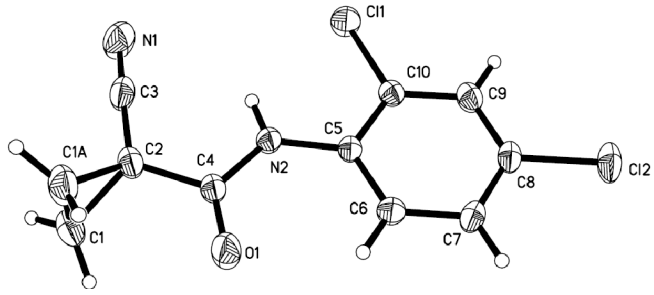


Fig. 2. Molecular Structure of the title compound

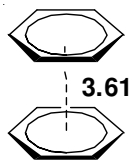


Fig. 3. Face-to-face π-π stacking

The X-ray analysis reveals that the benzene ring is planar. The carboxamide moiety is coplanar with the benzene ring [dihedral angle 180.0]. The inter-atomic distance for C(5)-O(2) is 1.205(6), which shows it is a normal C=O double bond. The conformation of the N-H bond in the NH-C(O) segment of the structure is anti to the C=O bond, similar to that observed in 1-cyano-*N*-(*p*-tolyl)cyclopropanecarboxamide. The phenyl ring is vertical with the cycloprane ring. The intermolecular face-to-face π-π stacking appears between the two phenyl ring in another adjacent molecule (Fig. 3), in which the distance of the centroid of phenyl ring is 3.61 Å. These interactions can help to further stabilize the crystal structure.

KARI activity: The primary bioassay shows the title compound exhibits a strong inhibiting activity towards KARI, which reaches 97.04 % at 200 μg/mL.

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